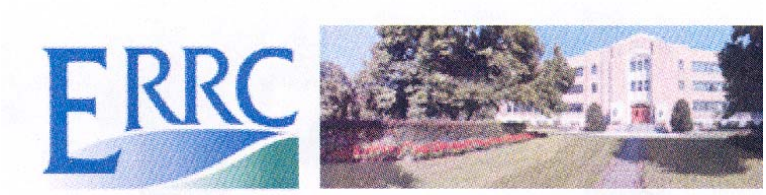

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Title: Radiation Resistance of Virulence Plasmid-Containing and Plasmid-Less Yersinia Enterocolitica

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Citation: Journal of Food Protection (2002) 65(3): 556-559

Number: 7081

Research Note

Radiation Resistance of Virulence Plasmid-Containing and Plasmid-Less *Yersinia enterocolitica*†

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MS 01-181: Received 18 May 2001/Accepted 10 September 2001

ABSTRACT

Yersinia enterocolitica, a foodborne pathogen, can be eliminated from meat by ionizing radiation. *Y. enterocolitica* sometimes contains a 70-kb virulence plasmid that encodes genes for a type III secretion channel and host immune suppression factors. The radiation resistance of virulence plasmid-containing and plasmid-less *Y. enterocolitica* was determined. Four *Y. enterocolitica* serotypes containing (i) the large virulence plasmid, and (ii) their plasmid-less derivatives were inoculated into raw ground pork, which was then vacuum packed and irradiated at 4°C to doses of 0.2, 0.4, 0.6, 0.8, and 1.0 kGy. The D_{10}-value, the radiation dose required to reduce the number of viable *Y. enterocolitica* by 90%, was not dependent on the presence or absence of the virulence plasmid, but it did differ among the four *Y. enterocolitica* serotypes.

Yersinia enterocolitica causes an estimated 96,000 cases of food-related illness in the United States annually *(13)*. It is considered to be a pathogen of concern by the pork-processing industry in the United States and is easily isolated from retail pork products *(5, 14)*. It can grow under refrigeration conditions and in high salt environments *(19)*. The virulence of *Y. enterocolitica* is linked to the presence of a 70-kb virulence plasmid that encodes genes for a type III secretion channel and host immune suppression *(4)*. Although many studies have investigated the incidence rates of *Y. enterocolitica* contamination in pork and other retail food products, the virulence status (the presence or absence of the large virulence plasmid) of *Y. enterocolitica* has not been described.

Refrigerated red meats, including pork, can be pasteurized by ionizing radiation doses up to 4.5 kGy *(6)*. Ionizing radiation can eliminate *Y. enterocolitica* from refrigerated pork products *(7, 10, 16, 17)*. However, to our knowledge, no study that directly compares the radiation resistance of plasmid-containing *Y. enterocolitica* with plasmid-less *Y. enterocolitica* has been conducted. The purpose of this study was to determine and compare the D_{10}-values, the radiation dose required to reduce the number of viable microorganisms by 90%, of virulence plasmid-containing and plasmid-less *Y. enterocolitica* of different serotypes.

MATERIALS AND METHODS

Pork. Raw pork roast was purchased from a local market and ground through a 3.1-mm grinder plate. The ground pork (20% fat) was then aliquoted (100 g) into no. 400 stomacher bags (Tekmar, Inc., Cincinnati, Ohio) and vacuum packed to 0.26 mm Hg with a Multi-Vac A300 Vacuum-Packager (Kansas City, Mo.). Next, to eliminate contaminating microorganisms, the meat was sterilized *(21)* by irradiating to a dose of 42 kGy (−30°C). The meat was then stored at −70°C until ready for use.

Strains. Four *Y. enterocolitica* strains (S. Weager, Food and Drug Administration, Seattle, Wash.) containing (i) the 70-kb

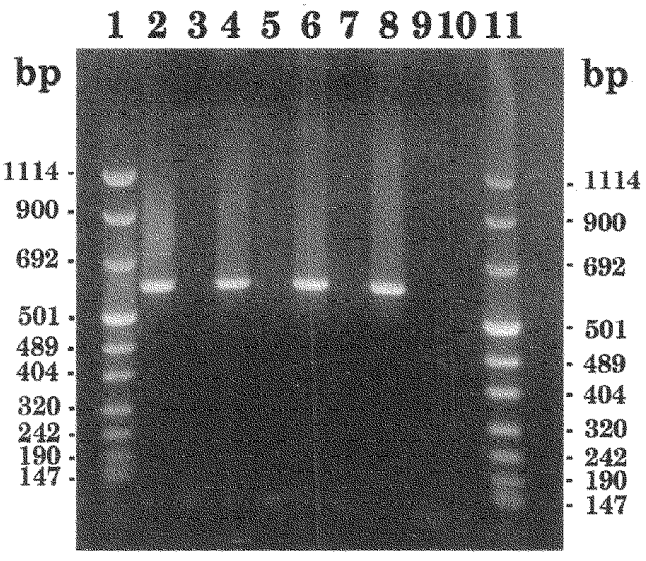

FIGURE 1. *Polymerase chain reaction amplification of 591 bp of the virulence plasmidborne* VirF *gene from plasmid-containing (P+) and plasmid-less (P−)* Y. enterocolitica *strains. Lane 2, GER P+; lane 3, GER P−; lane 4, ATCC 51871 (P+); lane 5, ATCC 51871 (P−); lane 6, PT18-1 (P+); lane 7, PT18-1 (P−); lane 8, EWM5 (P+); lane 9, EWM5 (P−); lane 10, primer (no template) control; lanes 1 and 11, molecular weight markers.*

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

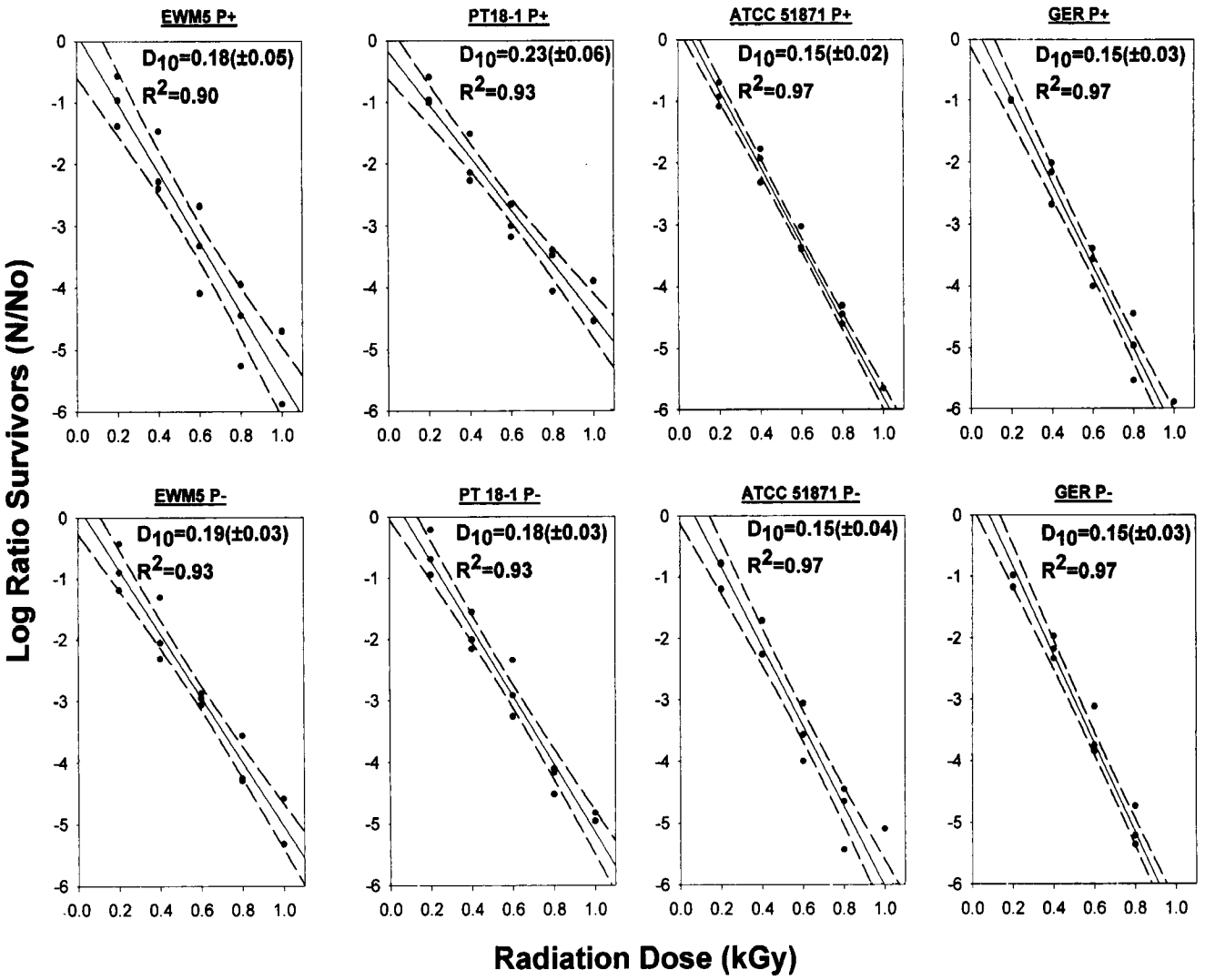

FIGURE 2. *Radiation resistance of* Y. enterocolitica *isolates suspended in raw ground pork. Linear regressions are shown as solid lines, and 95% confidence intervals are shown as dashed lines. Each experiment was conducted independently three times.*

large virulence plasmid, and (ii) their plasmid-less derivatives, i.e., GER (serotype O:3), ATCC 51871 (serotype O:8), PT18-1 (serotype O:5,27), and EWM5 (serotype O:13), were utilized. Strains were propagated on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) at 27°C and maintained at 0 to 2°C until ready for use. Species verification was performed by gram-negative identification cards with the Vitek Automicrobic System (bioMerieux, Inc., Hazelwood, Mo.). Presence of the virulence plasmidborne *VirF* gene was verified by polymerase chain reaction *(2).* Presence of the large virulence plasmid was also verified by plasmid DNA isolation and visualization via agarose gel electrophoresis (data not shown).

D_{10}-values. The *Y. enterocolitica* strains were cultured independently in 100 ml brain heart infusion medium (Difco) with baffled 500-ml Erlenmeyer flasks at 27°C (150 rpm) for 18 h. The bacteria were then sedimented by centrifugation (4°C, 1,725 × *g*) and resuspended as a cocktail in a 10-fold reduced volume of Butterfield's phosphate buffer (Applied Research Institute, Newtown, Conn.). The individual strains were then diluted 1/10 into 100 g sterile ground pork and mixed for 90 s in a Stomacher Mixer (Tekmar). The inoculated pork was then aliquoted (5 g) into no. 400 stomacher bags, vacuum packed to 0.26 mm Hg, and refrigerated for 15 to 30 min until irradiation.

A Lockheed Georgia Company (Marietta, Ga.) self-contained ^{137}Cs irradiator was used for all exposures. The radiation source consisted of 23 individually sealed source pencils in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was located central to the array when placed in the operating position. Inoculated samples were placed vertically and centrally in the sample chamber, using a 4-mm-thick polypropylene bucket, to ensure dose uniformity. The dose rate was 0.10 kGy/min.

The temperature during irradiation was maintained at the target by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber. Temperature was monitored by two thermocouples, one placed centrally in the chamber and the other taped to the side of the sample bag. The absorbed dose was verified with 5-mm alanine pellets that were measured by a Bruker EMS 104 EPR Analyzer (Billerica, Mass.). The ionizing radiation doses were 0.2, 0.4, 0.6, 0.8, and 1.0 kGy.

After irradiation, the samples were then assayed for colony-forming units by the standard pour plate method with brain heart infusion agar and 1/10 serial dilutions in Butterfield's phosphate buffer. Plates (three per dilution) were incubated at 37°C for 1 day before scoring. Colony-forming units per plate (30 to 300) were scored with a new Brunswick Scientific Biotran II colony counter. Unirradiated controls were routinely tested for the virulence plas-

TABLE 1. *Pairwise comparisons of* D_{10}*-values for plasmid-containing* Y. enterocolitica

Temp	EWM P+	PT18-1 P+	GER P+	51871 P+
EWM P+	—			
PT18-1 P+	$P = 0.043$[a]	—		
GER P+	$P = 0.039$[a]	$P \leq 0.001$[b]	—	
51871 P+	$P = 0.294$	$P \leq 0.001$[b]	$P = 0.950$	—

[a] Significantly different as determined by analysis of covariance ($n = 3$, $\alpha = 0.05$).

[b] Significantly different as determined by analysis of covariance ($n = 3$, $\alpha = 0.01$).

mid-associated trait of crystal violet binding as well as for colony morphology *(1)*.

The means of triplicate plate counts of the treated samples (*N*) were divided by the average control plate counts (*No*) to give a survivor ratio (*N/No*). The $\log_{10}$ (*N/No*) of the ratios was then used for the determination of D_{10}-values and other statistical analyses. D_{10}-values were determined by the reciprocal of the slopes after linear regression by least-squares analysis *(18)* (version 5.0, Sigma Plot, Chicago, Ill.). The predictive equation for the determination of $\log_{10}$ survivor ratios was performed with Sigma Plot, version 5.0. Analysis of covariance was performed with Statistical Analysis Systems software, version 6.12 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Polymerase chain reaction amplification of the virulence plasmid-associated 591-bp *VirF* gene product from the four virulence plasmid-containing *Y. enterocolitica* strains used in the study (lanes 2, 4, 6, and 8) and from their plasmid-less derivatives (lanes 3, 5, 7, and 9) is shown in Figure 1. Growth of overnight cultures and inoculation of pork were equivalent as determined by analysis of variance ($n = 3$, $\alpha = 0.05$) and were independent of the presence of the virulence plasmid or serotype.

The radiation resistances of the *Y. enterocolitica* suspended in raw ground pork are shown in Figure 2. The D_{10}-values were consistent with those obtained in other studies *(7, 8, 10, 12, 16)*. Statistical analysis with analysis of covariance ($n = 3$, $\alpha = 0.05$) indicated that the D_{10}-values obtained were equivalent in plasmid-containing and plasmid-less strains. However, the D_{10}-values of PT18-1 (serotype O:5,27) and EWM5 (serotype O:13) were greater than those obtained for GER (serotype O:3) and ATCC 51871 (serotype O:8), regardless of virulence plasmid status. The results of pairwise comparisons are shown in Table 1.

D_{10}-values are defined as the radiation dose required to eliminate 90% of the offending microorganism. These values, although sometimes presented as absolutes, are actually estimates that contain variability and uncertainty. Thayer *(20)* reviewed factors that affect the radiation resistance of microorganisms, which include the genetic diversity within a bacterial species, the suspending medium (meat or culture medium), the product temperature, etc. Differences in radiation resistance of microorganisms within the same species are common *(3, 15, 21)* and were observed among the *Y. enterocolitica* serotypes (Table 1). No difference in the radiation resistance between plasmid-containing and plasmid-less isolates of the same serotype was observed.

Most studies of *Y. enterocolitica* incidence in retail meats do not address the question of virulence plasmid status *(9, 11, 12)*. Previous estimates of *Y. enterocolitica* radiation resistance have not described the D_{10}-values of plasmid-containing versus plasmid-less isolates or the variations in radiation resistance between isolates *(7, 8, 10, 16)*. The D_{10}-values presented here will add information to the limited data base for the radiation resistance of *Y. enterocolitica* and will assist processors in selecting radiation doses for its safe and effective elimination from raw pork products.

ACKNOWLEDGMENT

We thank Laren Melenski for technical assistance.

REFERENCES

1. Bhaduri, S., L. Conway, and V. Lachica. 1987. Assay of crystal violet binding of virulent plasmid-bearing clones of *Yersinia enterocolitica*. J. Clin. Microbiol. 25:1039–1042.
2. Bhaduri, S., and B. Cottrell. 1997. Direct detection and isolation of plasmid-bearing virulent serotypes of *Yersinia enterocolitica* from various foods. Appl. Environ. Microbiol. 63:4952–4955.
3. Buchanan, R., S. Edelson, K. Snipes, and G. Boyd. 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. Appl. Environ. Microbiol. 64:4533–4535.
4. Cornelis, G. R., A. Boland, A. P. Boyd, C. Geuijin, M. Iriarte, C. Neyt, M. Sory, and I. Stanier. 1998. The virulence plasmid of *Yersinia*, an antihost genome. Microbiol. Mol. Biol. Rev. 62:1315–1352.
5. Davies, P. 1997. Food safety and its impact on domestic and export markets. Swine Health Prod. 5:13–20.
6. Federal Register. 1997. Irradiation in the production, processing, and handling of food. Fed. Regist. 62:64107–64121.
7. Grant, I. R., and M. F. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbiological and sensory quality of pork stored at refrigeration temperatures. Int. J. Food Sci. Technol. 26:507–519.
8. Grant, I. R., and M. F. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbiological safety of minced pork stored under temperature abuse conditions. Int. J. Food Sci. Technol. 26:521–533.
9. Hanna, M. O., D. L. Zink, Z. L. Carpenter, and C. Vanderzant. 1976. *Yersinia enterocolitica*-like organisms from vacuum-packaged beef and lamb. J. Food Sci. 41:1254–1256.
10. Kamat, A. S., S. Khare, T. Doctor, and P. M. Nair. 1997. Control of *Yersinia enterocolitica* in raw pork and pork products by γ irradiation. Int. J. Food Microbiol. 36:69–76.
11. Kwaga, J. K., and J. O. Olson. 1991. Laboratory investigation of virulence among strains of *Yersinia enterocolitica* and related species from pigs and pork products. Can. J. Microbiol. 38:92–97.
12. Mattila, T., and A. J. Frost. 1988. The growth of potential food poisoning organisms on chicken and pork muscle surfaces. J. Appl. Bacteriol. 65:455–461.
13. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.
14. Myers, B. R., R. T. Marshall, J. E. Edmonson, and W. C. Stringer. 1982. Isolation of pectinolytic *Aeromona hydrophila* and *Yersinia enterocolitica* from vacuum-packaged pork. J. Food Prot. 45:333–337.
15. Niemira, B. A., C. H. Sommers, and G. Boyd. 2001. Irradiation inactivation of four *Salmonella* serotypes in orange juice with various turbidities. J. Food Prot. 64:614–617.
16. Shenoy, K., E. Murano, and D. Olson. 1998. Survival of heat-

shocked *Yersinia enterocolitica* after irradiation in ground pork. Int. J. Food Microbiol. 39:133–137.
17. Sommers, C., and S. Bhaduri. 2001. Loss of crystal violet binding activity in *Yersinia enterocolitica* following gamma irradiation. Food Microbiol. 18:367–374.
18. Statistical Analysis Systems Institute (SAS). 1987. SAS/STAT guide for personal computers, version 6. SAS Institute, Inc., Cary, N.C.
19. Sutherland, J. P., and A. J. Bayliss. 1994. Predictive modelling of growth of *Yersinia enterocolitica:* the effects of temperature, pH, and sodium chloride. Int. J. Food Microbiol. 21:197–215.
20. Thayer, D. 2000. Sources of variation and uncertainty in the estimation of radiation D-10 values for foodborne pathogens. ORACBA (Office of Risk Assessment and Cost-Benefit Analysis) News 5:1–4.
21. Thayer, D., G. Boyd, C. Muller, W. Lipson, W. Hayne, and S. Baer. 1990. Radiation resistance of *Salmonella.* J. Ind. Microbiol. 5:373–390.